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Diethanolamine-Carbon Dioxide Buffer Produces Ethylene

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ABSTRACT

Carbon dioxide concentrations in containers are frequently controlled by using a diethanolamine-bicarbonate buffer. Current studies show that this buffer produces ethylene and that the production increases with increasing pH and/or time in the incubation vessel. Ethylene is not produced when 3-amino-1-propanol is used instead of diethanolamine in the buffer solution. The modified buffer is equally or more effective in controlling the CO_2 concentrations around plants incubated in the light or dark than the diethanolamine buffer.

INTRODUCTION

Carbon dioxide concentration (partial pressure) must be controlled in many experiments with plants and animals. Pardee (1949) proposed a buffer of diethanolamine and $KHCO_3$ to control CO_2 concentrations. The pH was adjusted with concentrated HCl to achieve the desired CO_2 concentration. Krebs (1951) presented the theory of CO_2 buffers and suggested adding thiourea to the Pardee buffer as an anti-oxidant. Preliminary experiments in our laboratory with the diethanolamine-CO_2 buffer indicated that ethylene was produced as a by-product of the buffering action. Low concentrations of ethylene can influence plant growth, development and biochemical processes (Abeles, 1973). Experiments were performed to determine the magnitude of the ethylene production by the diethanolamine buffer and to test a primary hydroxylamine, 3-amino-1-propanol, as an alternative to diethanolamine in the CO_2 buffer.

MATERIALS AND METHODS

The CO_2 buffer of Pardee using diethanolamine (2,2-dihydroxydiethylamine) (4 M) was prepared and equilibrated as described by Umbreit (1964). The pH was adjusted with concentrated HCl to produce the desired CO_2 concentrations. Prior to using the diethanolamine it was placed in a boiling flask and purged for 1 h with hydrocarbon-free nitrogen (flow rate 1·5 l min^{-1}) to remove possible volatile organic contaminants. After 24 h equilibration at 22 °C, 2 ml buffer solutions were transferred to 125 ml flasks and sealed with serum stoppers and maintained at 22 °C. Carbon dioxide and ethylene were measured at various intervals up to 24 h. When preparing an alternative buffer, the diethanolamine was replaced with an equal volume of 3-amino-1-propanol (5 M). Measurements indicated that none of the equipment used in the investigation outgassed ethylene.

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An experiment was conducted with plant tissue to test the ability of the buffer to control the gas phase CO_2 concentration in the incubation vessels. Chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.) plants were grown in a greenhouse as described by Tingey, Pettit, and Bard (1978). Leaves (approximately 15–17 cm^2) were detached from the plants and placed in 125 ml flasks containing a gauze wick inserted in a vial holding 4 ml buffer. The flasks were sealed with serum stoppers and incubated in the light or dark for 1 h at 26 °C.

Samples for gas chromatographic analysis were collected from the incubation flasks using 1 ml glass gas-tight syringes. Carbon dioxide was separated isothermally (95 °C) on a Porapak Q column (0·3 cm × 365 cm) using a helium carrier gas flow of 30 cm^3 min^{-1} and measured with a thermal conductivity detector. Ethylene was separated and measured with a flame ionization detector as previously described (Tingey *et al.*, 1978). The presence of ethylene in the samples was confirmed by co-chromatography with standard ethylene on a Porapak N column and by absorption of ethylene in bromine water (Crippen, 1973).

The studies indicated that the variance of the ethylene and CO_2 measurements increased with the mean concentration of the gas. Therefore, the data were transformed to their respective natural logarithms to stabilize the variability (Snedecor and Cochran, 1968). All statistical and regression analyses were performed on the transformed data. Anti-logs were taken to simplify presentation of the final results.

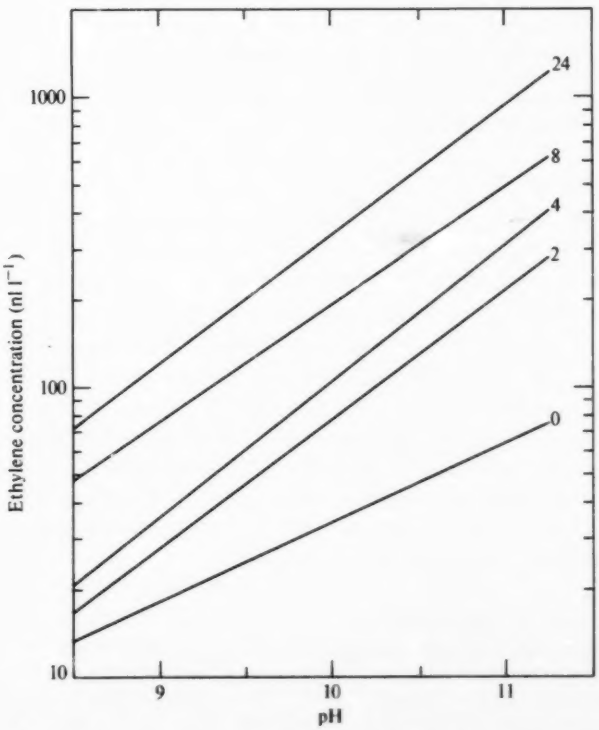

FIG. 1. The influence of pH on ethylene production from the diethanolamine-CO_2 buffer in an aerobic environment. Each linear regression line is based on 30 observations over 6 pH levels. The percent variation (R^2) explained by the linear model varied between 0·87 and 0·90 except at time zero which was 0·57. Numbers on the graph indicate the time in hours since the buffer was introduced into the incubation vessels.

TABLE 1. *Effect of thiourea on ethylene production in an anaerobic environment*[a]

Thiourea concentration (mM)	Ethylene concentration[b] ($nl\ l^{-1}$)
0	11·5 (1·3)
23	104·0 (1·3)
47	80·4 (1·2)

[a] The Pardee buffer was prepared according to Umbreit (1964) and the pH adjusted to 11·25. The buffer was maintained at 22 °C in a nitrogen atmosphere for 24 h when ethylene was measured.
[b] The ethylene concentrations shown are the geometric means and the numbers in parentheses are the standard geometric deviations based on 3 observations each.

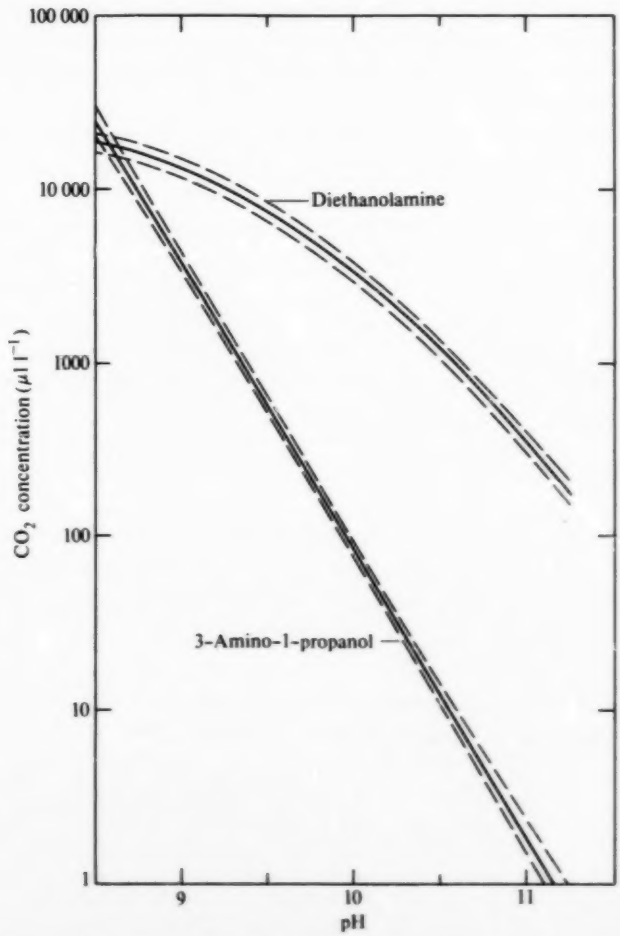

FIG. 2. The comparison of two alkanol amine buffer solutions on the CO_2 concentration as a function of pH. Each regression line is based on 30 observations taken at 6 pH levels. The regression lines are shown with their respective 95% confidence intervals.

RESULTS

Initial studies with the diethanolamine buffer were conducted to determine the relationship between the buffer solution pH, time, and ethylene production. Ethylene production increased exponentially with increasing pH (Fig. 1). Production increased about 40% per pH unit at all time intervals except 0 h where the increase was about 30%. The significant increase in ethylene detected at 0 h (immediately after sealing the flasks) indicated a rapid release of ethylene from the solutions. Ethylene production at all pH values increased by approximately 20% h^{-1} up to 8 h, after which it increased at a slower rate. Similar results were obtained using two different lots of diethanolamine and with diethanolamine that had not been purged with hydrocarbon-free nitrogen.

Studies were also conducted on the diethanolamine buffer in a nitrogen atmosphere (Table 1). Ethylene was produced in an anaerobic environment but the rate of production was substantially less than in an aerobic environment (Fig. 1). The addition of thiourea, as an anti-oxidant, to the buffer markedly stimulated ethylene production.

With verification that the diethanolamine-CO_2 buffer produced ethylene when prepared as described (Pardee, 1949; Krebs, 1951; Umbreit, 1964) a primary hydroxylamine was tested for use in the CO_2 buffer. 3-Amino-1-propanol gave satisfactory control of CO_2 concentrations when compared to diethanolamine (Fig. 2). At pH 8·5 and 22 °C both buffers produced essentially the same CO_2 concentration. However, 3-amino-1-propanol decreased the CO_2 concentration more rapidly with increasing pH than did diethanolamine. At pH 11 the CO_2 concentrations were 350 and 2 $\mu l\ l^{-1}$ for diethanolamine and 3-amino-1-propanol, respectively. With 3-amino-1-propanol there was no detectable production of ethylene or other C_2 or C_3 paraffins or olefins.

A study was conducted to test the ability of the 3-amino-1-propanol to liberate or absorb CO_2 as needed by plant tissue (Table 2). Incubation of chrysanthemum leaves in the light, without a CO_2 buffer decreased the CO_2 concentration considerably, while incubation in the dark without the CO_2 buffer caused a large increase in the CO_2 concentration. However, the CO_2 concentrations around leaves incubated in flasks containing the buffer were maintained near ambient levels, 365 and 353 $\mu l\ l^{-1}$ in the light and dark, respectively.

TABLE 2. *Control of the ambient CO_2 concentration ($\mu l\ l^{-1}$) under various incubation conditions using the 3-amino-1-propanol buffer*[a]

Treatment	Incubation conditions	
	Light	Dark
CO_2 buffer	365·2 (1·11)	352·9 (1·06)
No CO_2 buffer	16·7 (1·16)	2401·4 (1·20)

[a] The CO_2 concentration shown are the geometric means and the numbers in parentheses are the standard geometric deviation based on 18 observations each. The chrysanthemum leaf areas ranged between 15 and 17 cm^2. Plants were incubated for 1 h at 26 °C before measuring CO_2.

DISCUSSION

The use of the diethanolamine buffer at pH values between 10·5 and 11·25 yielded CO_2 concentrations from subambient to approximately three times ambient CO_2 concentration. Over this range of CO_2 concentrations there was significant ethylene production. The ethylene concentrations ranged from a low of 40 nl l^{-1} at zero time to a high of 400 nl l^{-1} after 4 h incubation. These levels of ethylene are sufficient to influence ethylene-sensitive processes (Abeles, 1973). In contrast, the 3-amino-1-propanol buffer produced no ethylene or other C_2 or C_3 contaminants. It controlled the CO_2 production and consumption by plant tissue and controlled CO_2 over a wide concentration range. 3-Amino-1-propanol is readily soluble in water but has a lower boiling point than diethanolamine, suggesting that it may be more volatile. The K_b of 3-amino-1-propanol is $1{\cdot}17 \times 10^{-4}$ (Schwabe, Graichen, and Spiethoff, 1959) which is similar to the value Krebs (1951) recommended for use in CO_2 buffers.

Ethylene can be produced by a range of chemicals and laboratory equipment including autoclaved rubber stoppers, ballast chokes for fluorescent lights, and autoclaved agar media containing organic growth compounds (Abeles, 1973). The greater ethylene production in an aerobic than an anaerobic environment is similar to the oxygen requirement of ethylene production *in vivo* (Liberman, 1979). Washio and Kiguchi (1975) have patented the reaction between ethanolamine, diethanolamine, or triethanolamine and organic acids as an accelerating agent for fruit ripening. They suggested that ethylene was the active agent in the process and have reported similar responses with organic amines and mineral acids (Washio and Kiguchi, 1973). The function of thiourea as a defoliant (Addicott and Carns, 1964) may result from its ability to stimulate ethylene production. The results of our study indicate that care should be used in selecting CO_2 buffers to insure that by-products such as ethylene are not produced which may inadvertently influence the outcome of experiments.

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